CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Oct. 2005, p. 1246–1250 Vol. 12, No. 10
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.10.1246–1250.2005

Humoral and Cellular Immunity in Children with *Mycoplasma pneumoniae* Infection: a 1-Year Prospective Study

Iwona Stelmach,[1]* Malgorzata Podsiadłowicz-Borzęcka,[1] Tomasz Grzelewski,[1] Pawel Majak,[1] Wlodzimierz Stelmach,[2] Joanna Jerzyńska,[1] Marta Popławska,[3] and Jaroslaw Dziadek[3]

M. Curie Hospital, Department of Pediatrics and Allergy, Zgierz, Poland[1]; Regional Health Insurance Fund, Lodz, Poland[2]; and Center for Medical Biology, Polish Academy of Sciences, Lodz, Poland[3]

Received 1 April 2005/Returned for modification 18 June 2005/Accepted 25 July 2005

To determine whether children have persistent abnormalities in cellular and humoral immunity development after acute *Mycoplasma pneumoniae* infection, serum immunoglobulin G (IgG), IgA, IgM, and IgE levels and lymphocyte phenotypes were determined. There were no changes in the levels of IgG, IgM, IgA, or $CD4^+$ or $CD19^+$ lymphocytes that were measured in *M. pneumoniae*-positive patients after 3 months or after 12 months, but there were increases in these in *M. pneumoniae*-negative patients. Serum IgE increased in *M. pneumoniae*-positive patients. We have shown alterations in immunity development after *M. pneumoniae* infection.

Mycoplasma pneumoniae is a common pathogen of children's respiratory tracts (8), and its abilities to act as a polyclonal activator of lymphocytes and autoantibodies to various tissues and immune complexes are well known (2, 16). $CD4^+$ T cells, B cells, and plasma cells infiltrate the lungs, which is followed by further amplification of the immune response, namely, proliferation of lymphocytes, production of immunoglobulins, and release of proinflammatory cytokines (3, 14). It has been previously described that the levels of total immunoglobulins, immunoglobulin A (IgA), IgM, and IgG, in serum increase during the convalescent phase of the disease (19) and that there is production of IgE specific to *M. pneumoniae* during infection (18). The bronchoalveolar lavage cytokine data suggest a predominant Th2-like cytokine response in *M. pneumoniae* infections, thus representing a favorable condition for IgE production (7), although other results suggest a Th1 cytokine response predominance (5, 21).

Previous studies are confined to the acute phase of *M. pneumoniae* infection and do not answer questions about the possible duration of the humoral and cellular imbalance after *M. pneumoniae* infection in children. In this study, we hypothesized that children may have persistent abnormalities in cellular and humoral immunity development after acute *M. pneumoniae* infection.

The study participants included 110 patients (52 male and 58 female) aged 1 to 5 years, all suffering from recurrent respiratory tract infections, defined according to Ribeiro (15). The diagnosis of *M. pneumoniae* infection was based on clinical symptoms (12, 20) and the presence of IgM, determined by enzyme-linked immunosorbent assay (ELISA) and confirmed by PCR. Children diagnosed with *M. pneumoniae* infection were treated with clarithromycin (4). None of the patients had previously suffered from allergic disease or immunodeficiency syndrome. The characteristics of the patients are presented in Table 1.

There were five study visits. At the first visit, patients were informed about the purpose of the study and were told that the second visit would occur after 3 months or earlier (in the case of respiratory tract infection). The patient's medical history was recorded, a physical examination was done, and blood samples for IgG, IgA, IgM, and IgE serum levels and lymphocyte phenotypes were taken at each visit. During the second visit, a blood sample was taken to determine the presence of *M. pneumoniae*-specific IgM by ELISA. For patients who had *M. pneumoniae* IgM, the third visit occurred 1 week after the second, for determination of *M. pneumoniae* DNA by PCR. For patients without *M. pneumoniae* IgM, the third visit occurred 3 weeks after the second, when a blood sample was collected for the second determination of *M. pneumoniae*-specific IgM by ELISA. The fourth visit was 3 months after the second, and the fifth visit was 12 months after the second.

Blood samples (5 ml) were collected, serum specimens were stored frozen, and the acute- and convalescent-phase serum specimens from each patient were tested for IgM antibodies in the same run. Upon their receipt, serum samples were serologically investigated for *M. pneumoniae*-specific IgM antibodies, using a commercial ELISA kit (Viro-Immun Labor-Diagnostica, Germany). We used capillary PCR to diagnose *M. pneumoniae* infection (6). Two whole-blood samples (3 ml) were obtained from each patient and were collected into sterile sodium heparinized tubes.

The presence of *Mycoplasma* DNA in the clinical samples collected was tested using a nested-PCR assay with primers MPP-11, MPP-12, and MPSW-1 (TGCCATCAACCCGCG

* Corresponding author. Mailing address: M. Curie Hospital, Department of Pediatrics and Allergy, 35 Parzeczewska Str., 95-100 Zgierz, Poland. Phone: (48 42) 716 49 82. Fax: (48 42) 716 49 82. E-mail: interna@wss.zgierz.pl.

TABLE 1. Patient baseline characteristics

Characteristic	Value for patient group		*P*
	MP positive[a] ($n = 24$)	MP negative[b] ($n = 15$)	
No. of males	12	7	
Age (yr) (mean ± SD)	4.1 ± 1.1	4.5 ± 1.4	0.84
Median no. of episodes of respiratory tract infection/yr (range)	6 (3–12)	7 (4–12)	0.74
Median no. of courses of antibiotics per yr (range)	7 (4–14)	6 (4–15)	0.69

[a] Children with *M. pneumoniae* infection.
[b] Children without *M. pneumoniae* infection.

CTTAAC, CCTTTGCAACTGCTATAGTA, and CAAACC GGGCAGATCACCTTT, respectively). The target sequence for the amplification process was the 466-bp segment of the P1 cytadhesin gene. PCR mixtures were initially incubated at 95°C for 15 min. Next, 35 cycles of amplification were carried out, each consisting of three 1-min incubation periods at temperatures of 94°C, 55°C, and 72°C. The final elongation step was 15 min at 72°C. The amplification products were analyzed in 2% agarose gels and visualized by ethidium bromide staining. To control the quality of isolated DNA, all PCR-negative samples were enriched with *Mycoplasma* DNA and reamplified to confirm that the negative results were not due to inhibition of *Taq* polymerase activity. Moreover, control PCRs, with primers amplifying the human β-actin gene, were also performed. Negative control reactions, without DNA, were also included in each experiment. Total IgE levels were measured by using a Pharmacia CAP kit (Uppsala, Sweden). An immunoturbidimetric assay for the quantitative determination of IgG, IgM, and IgA in human serum with a Hitachi 912 clinical analyzer (Roche, France) was used, and the results were expressed in mg/dl. Lymphocyte phenotypes were determined with a FACSCalibur flow cytometer (Becton, Dickinson and Company, NJ) and direct conjugate two-color labeled monoclonal antibodies and were expressed as percentages of converted lymphocytes.

Normal reference ranges by age for all immunoglobulin levels in children were supplied by the manufacturers that provided the reagents (Pharmacia, Uppsala, Sweden, and Roche, France) (normal reference ranges for lymphocytes are included in reference 9).

The results were analyzed according to well-known statistical methods with StatSoft Statistica for Windows, release 6.0 (StatSoft, Inc., Tulsa, OK). To compare differences between groups at baseline, the Student *t* test (mean age) and the Mann-Whitney test (number of respiratory tract infections and courses of antibiotics per year) were used. Before the analysis, all measured immunological parameters were transformed to the normal distribution and in the next step were analyzed using analysis of variance for repeated measures to compare changes within and between groups. *P* values of <0.05 were considered to be significant.

The study was approved by the Ethics Committee of the Medical University of Lodz. All parents or guardians and, if possible, children gave their written consent for participation in this study.

Thirty-nine children completed the study: 15 with PCR-confirmed *M. pneumoniae* infection (*M. pneumoniae* positive), and 24 children without *M. pneumoniae* infection (*M. pneumoniae* negative). The infections were confirmed by ELISA, with four measurements: at the first visit, then at 3 weeks, 3 months, and 12 months (Table 1). There were no significant differences between groups at baseline of all measured parameters. None of the patients had a total absence of any of immunoglobulin isotypes. We showed no significant changes in serum IgG, IgM, or IgA levels in patients from the *M. pneumoniae*-positive group after the 3- or 12-month follow-up. In contrast, in *M. pneumoniae*-negative patients, serum levels of IgG ($P < 0.001$), IgM ($P = 0.039$), and IgA ($P = 0.002$) significantly increased. Serum levels of IgE were significantly higher in patients with *M. pneumoniae* after 3 months ($P < 0.001$) and 12 months ($P = 0.041$) than in the *M. pneumoniae*-negative group (Fig. 1).

There were no significant changes in levels of $CD4^+$ and $CD19^+$ lymphocytes in patients from the *M. pneumoniae*-positive group after the 3- or 12-month follow-up. In *M. pneumoniae*-negative patients, all levels of analyzed lymphocyte phenotypes significantly increased ($P < 0.001$). There were no differences in $CD8^+$ and $CD3^+$ lymphocytes between groups (Fig. 2).

To the best of our knowledge, there are no data available on humoral and cellular immunity in children months after an acute *M. pneumoniae* infection. The dynamics and nature of serum-specific antibodies during acute *M. pneumoniae* infection have been thoroughly studied; most patients develop *M. pneumoniae*-specific IgG, IgM, IgA, and IgE (1, 18). However, regarding serum levels of total immunoglobulins, only one study revealed that the high levels of total immunoglobulins IgA, IgM, and IgG in serum can persist in the convalescent phase of the disease (19). *M. pneumoniae* strains are likely to induce transient anergy in most patients during the acute phase of infection by mechanisms yet to be clarified. An interesting question is whether this anergy is only a transient process or whether *M. pneumoniae* infections can cause protracted anergy of the immune system, especially in children, which may have important implications for their health status (continuation of recurrent infections) and treatment. The results of our study showed that after *M. pneumoniae* infection, there were no increases in the levels of the IgG, IgM, and IgA immunoglobulins during a 1-year observation compared to those of an *M. pneumoniae*-negative group. This may suggest that *M. pneumoniae* can temporarily suppress the immune system. Our results oppose the results of Shimizu et al., who found that serum IgA and IgM increase in the convalescent phase of the *M. pneumoniae* infection (19). The same study showed that the levels of total serum IgE were higher in the acute phase and then gradually decreased. However, there were health status

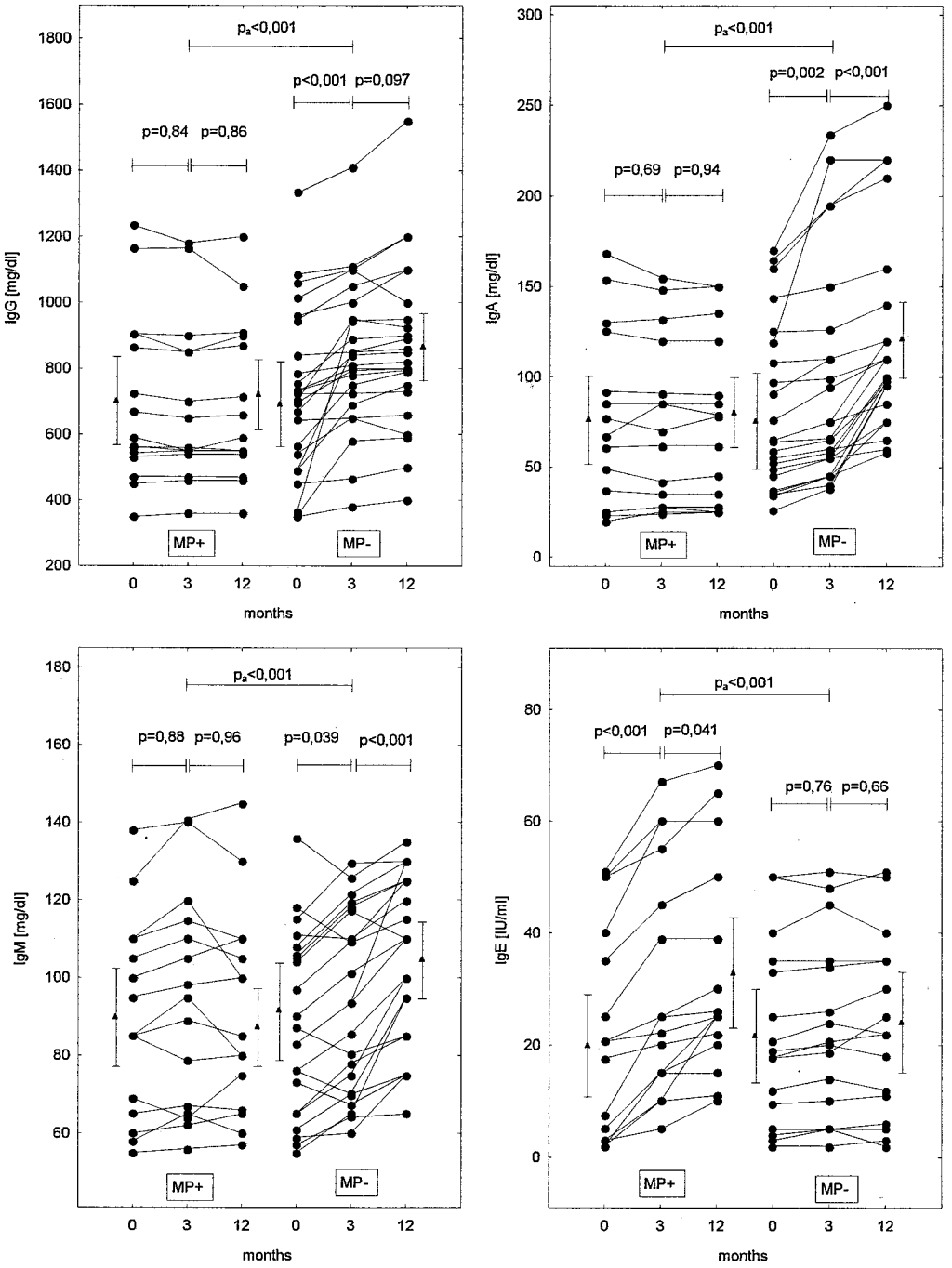

FIG. 1. Serum levels of IgG, IgA, IgM, and IgE in children with (MP+) and without (MP−) *Mycoplasma pneumoniae* infection. Mean *P* value changes during 12 months of follow-up (0 to 3 and 3 to 12 months) are given. Mean P_a (analysis of variance for repeated measures) value changes during 12 months of follow-up between groups are also shown.

differences in the children in the two studies: almost 50% of their patients had asthma, while our patients had no asthma but did have recurrent respiratory tract infections. These differences may explain the different results. Our total serum IgE results showed some agreement with those of other studies done after acute *M. pneumoniae* infection (10, 13, 18, 19) and also revealed a marked chronicity of total serum IgE increase. The phenomenon of total serum IgE increase may be explained by the development of *Mycoplasma*-specific IgE by patients during the acute phase of the disease (18), but we can only speculate on that.

We found that the percentage of $CD4^+$ and $CD19^+$ cells did not change 1 year after acute *M. pneumoniae* infection, while our *M. pneumoniae*-negative patients had a definitely higher percentage of $CD4^+$ and $CD19^+$ cells. Zhao et al. also showed that $CD4^+$ cells decreased at both the acute and recovery

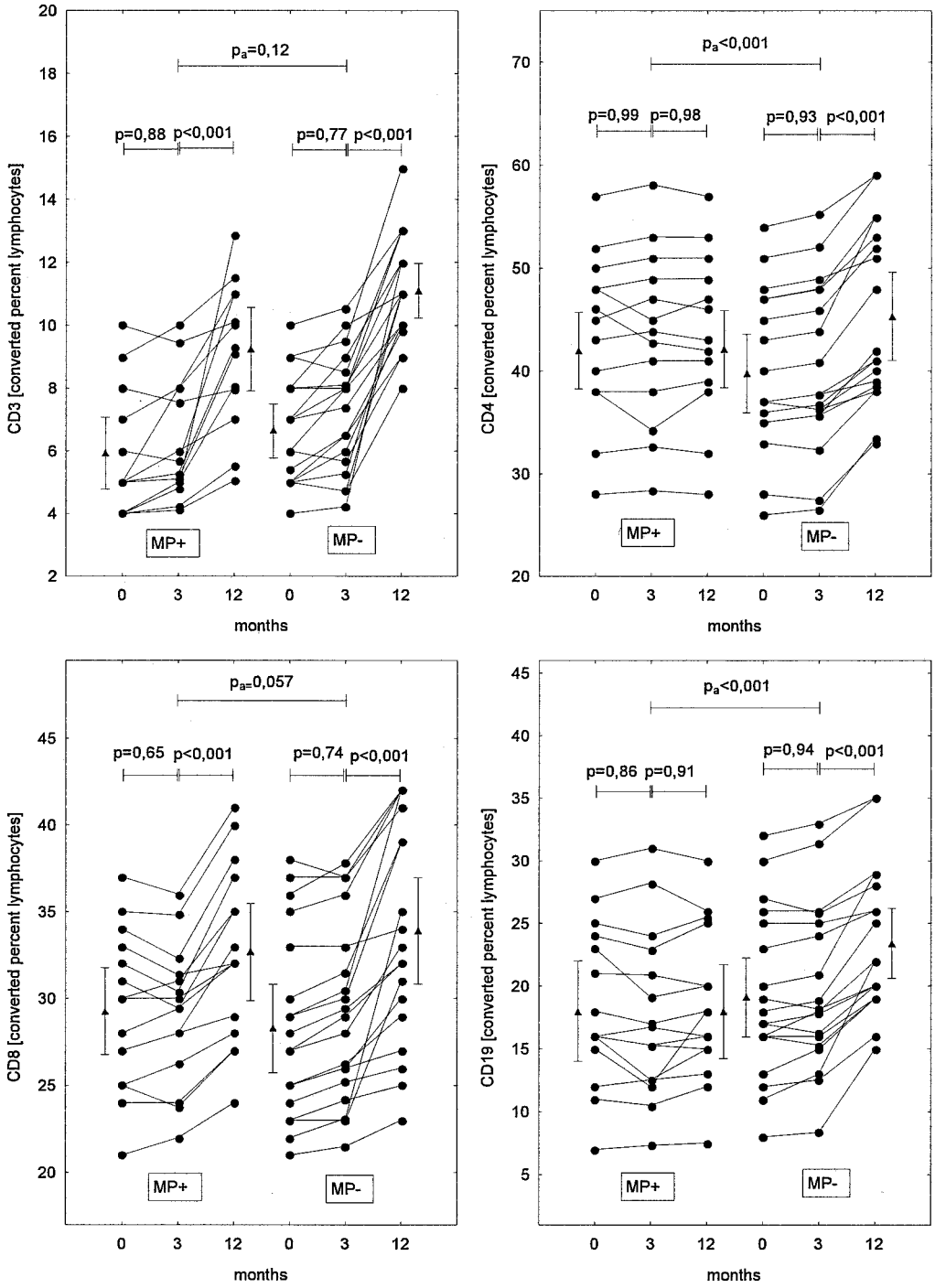

FIG. 2. Percentage of converted lymphocytes, i.e., $CD3^+$, $CD4^+$, $CD8^+$, and $CD19^+$ cells, in children with (MP+) and in children without (MP−) *Mycoplasma pneumoniae* infection. Mean *P* value changes during 12 months of follow-up (0 to 3 and 3 to 12 months) are given. Mean P_a value changes during 12 months of follow-up between groups (analysis of variance for repeated measures) are also shown.

stages of *M. pneumoniae* pneumonia (23). On the other hand, Hou et al. revealed an increase in the $CD4^+$ proportion of T lymphocytes in children with acute *M. pneumoniae* infection in comparison with that of healthy controls (7). Nevertheless, some studies showed that redistribution of $CD4^+$ T cells to the site of infection may be a reason for the decreased proportion of these cells in the blood (3, 11, 22).

Previous findings demonstrated that a high proportion of children suffering from recurrent infections have an immune "developmental delay" (17), but the role of pathogens in this phenomenon is still unclear. For this reason, our study included young children who were referred to our immunology clinic for recurrent infections.

Our study does have some limitations. Although healthy

children would have been the best control group, it would not have been acceptable from an ethical point of view to use healthy children in our study design because our study involved very young children. Instead, we cited a reference that included normal serum immunoglobulin and lymphocyte values for children. Another limitation of our study is the lack of respiratory virus testing. It is possible that *M. pneumoniae* infection acts as a cofactor, possibly rendering subjects more susceptible to other stimuli, such as viruses. Our data do not explain the possible ways by which *M. pneumoniae* suppresses the immune system, but that was not the aim of this study.

In summary, we have shown that there are some alterations in the development of humoral and cellular immune responses 1 year after acute *M. pneumoniae* infection in children. These alterations include changes in IgA, IgM, and IgG immunoglobulin levels and in $CD4^+$ and $CD19^+$ cells. However, further studies are needed to determine the duration of the immune response, to provide a more comprehensive understanding of how it affects the immune system in children, and to determine whether our results are generalizable to the majority of pediatric patients with *M. pneumoniae* infections.

REFERENCES

1. **Baizhomartov, M. S., I. K. Shuratov, V. A. Semenova, G. U. Diuskalieva, and O. G. Stetsenko.** 1985. Serum immunoglobulin in Mycoplasma pneumoniae infection. Zh. Mikrobiol. Epidemiol. Immunobiol. **2:**68–72. (In Russian.)
2. **Biberfeld, G., and R. Norberg.** 1974. Circulating immune complexes in mycoplasma infection. J. Immunol. **112:**413–415.
3. **Chan, E. D., and C. H. Welsh.** 1995. Fulminant Mycoplasma pneumoniae pneumonia. West. J. Med. **162:**133–142.
4. **Esposito, S., F. Blasi, C. Arosio, L. Fioravanti, L. Fagetti, R. Droghetti, P. Tarcia, L. Allegra, and N. Principi.** 2000. Importance of acute *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infections in children with wheezing. Eur. Respir. J. **16:**1142–1146.
5. **Fonseca-Aten, M., A. M. Rios, A. Mejias, S. Chavez-Bueno, K. Katz, A. M. Gomez, G. H. McCracken, Jr., and R. D. Hardy.** 2005. Mycoplasma pneumoniae induces host-dependent pulmonary inflammation and airway obstruction in mice. Am. J. Respir. Cell Mol. Biol. **32:**201–210.
6. **Honda, J., T. Yano, M. Kusaba, J. Yonemitsu, H. Kitajima, M. Masuoka, K. Hamada, and K. Oizumi.** 2000. Clinical use of capillary PCR to diagnose Mycoplasma pneumonia. J. Clin. Microbiol. **38:**1382–1384.
7. **Hou, A. C., Y. Lu, L. Sha, L. G. Liu, J. Shen, and Y. Xu.** 2003. T(H1) and T(H2) cells in children with mycoplasma pneumonia. Zhonghua Er. Ke. Zazhi **41:**652–656. (In Chinese.)
8. **Kaluzewski, S., M. Jasielski, W. Rastawicki, and M. Kochman.** 1994. Evaluation of occurrence of infections caused by *Mycoplasma pneumoniae* during 1970–1993 based on serological investigations. Przegl. Epidemiol. **48:**165–172. (In Polish.)
9. **Kawiak, J., R. Rokicka-Milewska, K. Zeman, G. Hoser, G. Derulska, E. Fornalczyk-Wachowska, B. Gosk, J. Kantorski, J. Pacholska, and H. Tchorzewski.** 1995. Peripheral blood leukocytes and lymphocyte subpopulations as determined by flow cytometric measurements in healthy children. Folia Histochem. Cytobiol. **33:**33–38.
10. **Koh, Y. Y., Y. Park, H. J. Lee, and C. K. Kim.** 2001. Levels of interleukin-2, interferon-gamma, and interleukin-4 in bronchoalveolar lavage fluid from patients with Mycoplasma pneumonia: implication of tendency toward increased immunoglobulin E production. Pediatrics **107:**E39.
11. **Llibre, J. M., A. Urban, E. Garcia, M. A. Carrasco, and C. Murcia.** 1997. Bronchiolitis obliterans organizing pneumonia associated with acute Mycoplasma pneumoniae infection. Clin. Infect. Dis. **25:**1340–1342.
12. **Marrie, T. J.** 1996. Atypical pneumonia revisited. Semin. Respir. Crit. Care Med. **17:**221–229.
13. **Nagayama, Y., and N. Sakurai.** 1991. Clinical observations on lower respiratory tract infections with special reference to serum IgE levels. Pediatr. Pulmonol. **11:**44–48.
14. **Opitz, O., K. Pietsch, S. Ehlers, and E. Jacobs.** 1996-1997. Cytokine gene expression in immune mice reinfected with Mycoplasma pneumoniae: the role of T cell subsets in aggravating the inflammatory response. Immunobiology **196:**575–587.
15. **Ribeiro, L. M., C. M. Jacob, A. C. Pastorino, C. A. Kim, A. B. Fomin, and A. P. Castro.** 2003. Evaluation of factors associated with recurrent and/or severe infections in patients with Down's syndrome. J. Pediatr. (Rio de Janeiro) **79:**141–148. (In Portuguese.)
16. **Ruuth, E., and F. Praz.** 1989. Interactions between mycoplasmas and the immune system. Immunol. Rev. **112:**133–160.
17. **Scornik, J. C., G. Elfenbein, J. Graham-Pole, T. Goedert, S. Gross, and R. S. Weiner.** 1992. Role of immunoglobulin subclasses and specific antibody determinations in the evaluation of recurrent infection in children. J. Pediatr. **121:**516–522.
18. **Seggev, J. S., G. V. Sedmak, and V. P. Kurup.** 1996. Isotype-specific antibody response to acute *Mycoplasma pneumoniae* infection. Ann. Allergy Asthma Immunol. **77:**67–73.
19. **Shimizu, T., H. Mochizuki, M. Kato, M. Shigeta, A. Morikawa, and T. Hori.** 1991. Immunoglobulin levels, number of eosinophils in the peripheral blood and bronchial hypersensitivity in children with Mycoplasma pneumoniae pneumonia. Arerugi **40:**21–27. (In Japanese.)
20. **Talkington, D. F., K. B. Waites, S. B. Schwartz, and R. E. Besser.** 2001. Emerging from obscurity: understanding pulmonary and extrapulmonary syndromes, pathogenesis, and epidemiology of human *Mycoplasma pneumoniae* infections, p. 57–84. *In* W. M. Scheld, W. A. Craig, and J. M. Hughes (ed.), Emerging infections, vol. 5. ASM Press, Washington, D.C.
21. **Tanaka, H., M. Narita, S. Teramoto, T. Saikai, K. Oashi, T. Igarashi, and S. Abe.** 2002. Role of interleukin-18 and T-helper type 1 cytokines in the development of *Mycoplasma pneumoniae* pneumonia in adults. Chest **121:** 1493–1497.
22. **Wang, L., K. C. Hong, F. C. Lin, and K. D. Yang.** 2003. Mycoplasma pneumoniae-associated Stevens-Johnson syndrome exhibits lymphopenia and redistribution of $CD4^+$ T cells. J. Formos. Med. Assoc. **102:**55–58.
23. **Zhao, H., L. Li, and X. Liu.** 1999. Cellular immunity and epidemiologic analysis of pediatric patients with Mycoplasma pneumonia. Zhonghua Liuxingbingxue Zazhi **20:**47–49. (In Chinese.)